PCT/GB 2003 / 0 0 2 3 6 9

The Patent Office

INVESTOR IN PEOPLE

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Stephen Hordley

Dated 4 July 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

JMH/6978

0212648.0

31 MAY 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Immunoclin Laboratories Ltd
Rowlandson House
289-293 Ballards Lane
London N12 8NP

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

8394314001

4. Title of the invention

"Treatment with Cytokines"

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Stevens Hewlett & Perkins
1 St. Augustine's Place
Bristol BS1 4UD
United Kingdom

Patents ADP number *(if you know it)*

1545002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	25
Claim (s)	2
Abstract	1
Drawing (s)	-

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. I/We request the grant of a patent on the basis of this application.

Signature Stevens, Hewlett & Perkins Date 31/05/02

STEVENS HEWLETT & PERKINS

12. Name and daytime telephone number of person to contact in the United Kingdom — Joanne Heaton 0117 922 6007

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

TREATMENT WITH CYTOKINES

This invention relates to the use of cytokines in the diagnosis, treatment or prophylaxis of diseases. More particularly, the present invention relates to the use of cytokines to diagnose or treat non-neoplastic or non-leukaemic diseases such as autoimmune diseases or neurodegenerative disorders.

In the description which follows, the present invention will be described with particular reference to the most preferred embodiment of the invention which relates to the use of the cytokine interleukin-10 in the diagnosis, treatment or prophylaxis of the neurodegenerative disorder Alzheimer's disease. It is not intended to restrict the scope of the present invention to this one embodiment since the present invention finds equal utility in other disorders such as autoimmune diseases, for example multiple sclerosis, myasthenia gravis, systemic lupus erythramatosus, diabetes mellitus and asthma, other neurodegenerative disorders for example Parkinson's disease, motor neurone disease and Alzheimer's disease; chronic inflammatory diseases such as rheumatoid arthritis; and other diseases where the modulation of T-Cell function is desirable such as HIV-infection and AIDS.

Similarly, the invention has utility with all cytokines, not solely interleukin-10 and hence it is intended to include cytokines such as interleukin-1 (α or β), interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, interleukin-17, interferon-α, interferon-β, interferon-γ, TNF-α, TNF-β, G-CSF, GM-CSF, M-LSF, and TGF-β, in the scope of the present invention.

The major cause of cognitive decline in the elderly is Alzheimer's disease (AD). Because of longer life spans worldwide, the number of people that will be affected by AD is estimated to triple over the next 50 years (1). AD is a clinical syndrome characterised by complex and heterogeneous pathogenic mechanisms. The recognised genetic factors include mutations of the gene encoding the amyloid precursor protein (2), presenilin 1 and 2 (3, 4), which account for a small part of familial and usually early-onset AD cases. Genetic

factors have been also associated with the sporadic or non familial form of the disease and the allele e4 of the apolipoprotein E (Apo E) significantly increases the risk of AD, but is neither necessary nor sufficient for the development of the disease (5- 7). Therefore other genetic and environmental factors are likely to be implicated and are actively investigated.

Molecules that take part in the inflammatory cascade are of great interest, because inflammation has repeatedly been suggested to be associated with the neurodegenerative process characteristic of the AD brain (8). Thus, reactive astrocytosis is observed both in the cortex and in the hyppocampus of these patients and the glial cells are also activated within or near the neuritic plaques. Over-expression of cytokines and other inflammatory molecules are common features of the AD brain pathology (9). Additionally, epidemiological studies showed that the long term use of non steroid anti-inflammatory drugs is associated with a decreased incidence of AD in a co-twin control study (10) and several other clinical studies confirmed a decreased association of AD in individual treated with anti-inflammatory drugs (11) including COX2 specific inhibitors (12). These findings support the hypothesis that inflammation might contribute to the neurodegeneration associated with AD (13).

In the attempt to better understand the biologic correlates of AD the possible role of several cytokines and chemokines has been recently investigated. Virtually all the mediators analyzed in these studies, including IL-1b, IL-6, TNF-a, IL-8, TGF-b and macrophage inflammatory protein-1a (MIP-1a), have been suggested to be up-regulated in AD compared to non demented controls (14- 18). On the contrary, conflicting results are obtained in relation to the immunomodulatory cytokine IL-10, a type-2 cytokine that suppresses T lymphocytes and cell-mediated immunity in humans and mice and has potent anti-inflammatory properties (19- 21).

The gene encoding IL-10, mapped to chromosome 1 between 1q31 and 1q32, is highly polymorphic. IL-10 production is correlated to biallelic polymorphisms at positions: -1082 (guanine to adenine substitution), -819 (timine to cytosine substitution), and -592 (adenine to cytosine substitution).

These allelic variations are associated with measurable differences in IL-10 production by antigen- and mitogen-stimulated peripheral blood lymphocytes. In fact these polymorphisms occur in the regulatory region of the gene and are associated with high, intermediate or low IL-10 production (22).

The present inventors investigated beta amyloid-stimulated IL-10 production by peripheral blood lymphocytes of AD patients and of age-matched healthy controls. Because the generation of this cytokine was significantly reduced in AD patients, IL-10 polymorphisms were analysed in these same individuals. Results showed that the high IL-10-producing allele is extremely rare in AD patients.

Specifically, IL-10 genotypes are differently distributed when AD are compared with HC (χ^2 = 16.007; p=0.007). Therefore genotypes corresponding to reduced IL-10 production have a significantly higher distribution amongst AD subjects (table I). The presence of low-IL-10-producing genotypes is associated with a worsened clinical outcome of AD as follows: 1) earlier age at disease onset (Table II); and 2) faster disease progression (MMSE score)(Table III).

Table I. IL-10 genotype distribution

Genotype (c)	AD n=47	HC n=25	AD %	HC %
GCC/GCC (H)	1	7	2	28
GCC/ACC (M)	10	9	21	36
GCC/ATA (M)	11	3	23	12
ACC/ACC (L)	8	1	17	4
ACC/ATA (L)	12	4	26	16
ATA/ATA (L)	5	1	11	4

The frequency of the different genotypes among Alzheimer's disease patients (AD) are statistically different from those of the health controls (HC). χ^2 = 16.007, df= 5, p= 0.007. In the brackets (c) there are the corresponding phenotype high (H), intermediate (M), low (L).

Table II. IL-10 genotype distribution and age at onset

Genotype	mean	S.D.	SEM
GCC/GCC	76	/	/
GCC/ACC	75.00	8.57	3.03
GCC/ATA	67.33	8.2	2.73
ACC/ACC	76.20	8.79	3.93
ACC/ATA	77.17	4.07	1.66
ATA/ATA	65.75	1.71	0.85

Correlation between the different genotypes in Alzheimer's disease patients and the age at onset. ANOVA: p= 0.042.

Table III. IL-10 genotype distribution and MMSE

Genotype	mean	S.D.	SEM
GCC/GCC	18		
GCC/ACC	21.75	5.5	1.94
GCC/ATA	16.33	5.68	1.89
ACC/ACC	10.80	7.5	3.35
ACC/ATA	13.83	5.19	2.12
ATA/ATA	22.5	1.73	0.87

Correlation between the different genotypes in Alzheimer's disease patients and MMSE ANOVA: p= 0.010.

Chronic inflammation is suggested to be involved in the neurodegenerative process characteristic of AD (24, 25); this suggestion stems from both *in vivo* and *ex adjuvantibus* criteria. Hence, inflammatory mediators and activated glial cells are observed to be closely associated with neuritic plaques *in vivo*. Furthermore, recent data indicate that anti-inflammatory therapy could be useful in modulating disease progression (10- 12). Despite this vast body of evidence, the biologic correlates of AD are still unclear. To shed light on this problem, focused attention was on

IL-10. This cytokine is a pivotal regulatory cytokine involved in many facets of the immune response and is dysregulated in human autoimmune (26), malignant (27- 31), and infectious (32- 35) diseases. More recently it has been shown that the presence of genetically-determined higher levels of IL-10 secretion is an important component of the genetic background to systemic lupus erythematosus (36) and to the outcome of infectious disease (37). It has also been demonstrated that IL-10 secretion, resulted from in vitro stimulation of human peripheral blood leukocytes with LPS, varies markedly between individuals and that cytokine haplotypes are associated with different secretion levels (38). In addition, differences in IL-10 serum production by cells of patients and of their first-degree family members (37, 39), as well as differences in the distribution of IL-10 alleles, suggested the involvement of the different isoforms of the IL-10 gene as an important quantitative trait loci for human disease in infections (37, 40), autoimmune (26, 36, 41, 42) and malignant disease (43).

The present inventors initially analyzed LPS-, Flu, and amyloid peptides- specific IL-2 and IL-10 production by peripheral blood mononuclear cells of AD patients and age matched HC. Results showed that: 1) IL-2 production by PBMC of AD patients and controls was similar in all the conditions measured; and 2) IL-10 generation by LPS- and Flu - stimulated PBMC was comparable similar amongst the two groups of individuals. In contrast, an amyloid-specific immune impairment characterized by a reduced generation of IL-10 was present in AD. The observation that this cytokine imbalance was not seen in mitogen-stimulated PBMC indicates that amyloid-specific immune responses are selectively impaired in AD patients. Additionally, results showing that flu-stimulated proliferation was similar in patients and controls indicates that antigenic processing and presentation in association with HLA class II molecules, and the CD4-HLA class II self-restricted pathway of activation of the immune system (44), are not defective in these patients.

Next polymorphisms were analyzed in the IL-10 gene in the same group of subjects. Results stemming from analysis of the distribution of

the IL-10 alleles in this Italian sample of healthy individuals showed a close similarity to those reported for other caucasic populations (45). In contrast, we observed a significantly higher frequency of the genotypes corresponding to reduced IL-10 production (ACC/ACC, ACC/ATA and ATA/ATA) in AD patients. Thus, an abnormally augmented prevalence of low-IL-10 producing isoforms in the AD population was determined; the phenotypic correlate of these isoforms becomes evident when amyloid-specific immune responses were measured.

Subsequent analyses focused on possible correlations between impaired IL-10 production and the clinical manifestations of AD by verifying whether the presence of low/intermediate IL-10 producing genotypes would be are associated with different disease outcomes. Results confirmed this to be the case. Thus, the presence of the ATA/ATA and of the GCC/ATA genotypes was correlated with an earlier age at disease onset. Additionally, the ACC/ATA and the ACC/ACC (all these are low/intermediate IL-10-producing genotypes) alleles were associated with a more severe cognitive impairment as indicated by a lower MMSE score.

It is interesting to observe that a recent report on Italian centenarians, individuals that -by definition- are less prone to develop age-related diseases, has demonstrated that extreme longevity is associated with a significantly higher frequence of the high IL-10-producing genotypes (46).

IL-10 is known to have potent antiinflammatory properties (47); a biological scenario could thus be hypothesized in which the reduction of amyloid-specific IL-10 production would favor the triggering of the chronic inflammatory process seen in the progression of AD. These results suggest that an amyloid-specific and IL-10-mediated inhibitory feed-back circuit may be active in non-AD individuals; the rupture of this circuit could be associated with/predictive for the development of AD. Recently, a convincing study showed that an IL-10/pro-inflammatory circuit that revolves around cells of the innate immune system regulates susceptibility

to autoimmune diseases (48). These results are expanded by showing that an alteration of this circuit is present in AD patients.

These data, support the role of inflammatory processes in the pathogenesis of AD; reinforce the hypothesis that in AD patients neurodegeneration is tightly associated with an aberrant antigen-specific immune response; and lend further support to the use of antiinflammatory compounds in the therapy of this disease.

Accordingly, the present invention provides a pharmaceutical composition comprising a cytokine in the preparation of a medicament for the treatment or prophylaxis of disease excluding neoplastic diseases, leukaemias, and acute inflammation. Preferably the disease is a neurodegenerative disorder or an autoimmune disease. Most preferably the disease is selected from the group comprising multiple sclerosis, myasthenia gravis, systemic lupus erythramatosus, diabetes mellitus, asthma, Parkinson's disease, motor neurone disease, Alzheimer's disease, chronic inflammation rheumatoid arthritis, HIV-infection and AIDS.

Preferably, the cytokine is selected from the group consisting of interleukin-1 (α or β), interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, interleukin-17, interferon-α, interferon-β, interferon-γ, TNF-α, TNF-β, G-CSF, GM-CSF, M-LSF, and TGF-β.

Most preferably the cytokine is an interleukin, especially interleukin-10.

In a further aspect of the invention polymorphic regions have been identified, which polymorphs are indicative of a dysfunction of cytokine production and hence are associated with a predisposition towards an autoimmune, neurodegenerative or chronic inflammatory disease.

Accordingly, in a second aspect the invention provides a method of determining a predisposition to Alzheimer's disease, autoimmune disease or other neurodegenerative diseases, the method comprising taking a DNA bearing sample from a subject animal, and analysing the sample for the presence or absence of the alleles of Figure 2.

In a further aspect the invention provides DNA fragments and cDNA fragments encoding the allelic polymorphs of Figure 2 for use in the above described method.

The invention also provides a method of treating Alzheimer's disease, autoimmune diseases or other neurodegenerative disorders by the modulation upregulation or downregulation of the gene of the allelic polymorphisms of Figure 2.

Embodiments of the invention will now be described by way of example only, with reference to the following examples.

Patients and controls

Forty-seven AD patients and 25 non-demented subjects (HC) were included in a study of Alzheimer's disease. These patients were selected from a larger population sample followed at the Geriatric Department of the Ospedale Maggiore IRCCS, University of Milan, Italy. The DMS IV and NINCDS-ADRDA (23) criteria were adopted to obtain the clinical diagnosis of AD. Cognitive performances and alterations were assessed according to the Mini-Mental State Evaluation (MMSE). AD patients and HC were living at home and were carefully physical examined on the day of blood collection and their clinical records evaluated. In order to minimize the risk of clinical or sub-clinical inflammatory processes, all the patients were selected as follows: only AD and HC without clinical sign of inflammation (e.g. normal body temperature or absence of concomitant inflammatory disease) were included in the study. Blood chemical parameters were also evaluated and subjects with abnormal sedimentation rate of red blood cells or altered blood profile of albumin and transferring plasma levels were excluded. A further selection of AD patients were performed according to the C reactive protein (CRP) plasma levels and those patients with CRP above 5 mg/l (mean value ± 2 standard deviations of control values) were not enrolled in the study.

Informed consent to perform the study was obtained from controls and a relative of each AD patient.

Blood sample collection

Whole blood was collected by venipuncture in Vacutainer tubes containing EDTA (Becton Dickinson Co, Rutherford, NJ). Peripheral blood mononuclear cells (PBMC) were separated by centrifugation on lymphocyte separation medium (Organon Teknika Corp., Durham, NC) and washed twice in PBS. The number of viable lymphocytes was determined by trypan blue exclusion and a hemocytometer.

In vitro cytokine production

PBMCs were resuspended at $3x10^6$/ml in RPMI 1640 and were either unstimulated or stimulated with LPS (Sigma, St. Louis, MI)(10 g/ml), with a pool of 3 different peptides from the b-amyloid protein as follows: b-A: fragment 25-35 (25 mg/ml); b-B: fragment 1-40 (150 ng/ml); b-C: fragment 1-16 (150 ng/ml) (Sigma, St. Louis, MI); or with influenza virus vaccine (A/Taiwan+A/Shanghai+B/Victoria)(24 g/l; final dilution 1:1000)(Flu)(control antigen) at 37°C in a moist, 7% CO_2 atmosphere. Supernatants were harvested after 48 hours for LPS stimulation and after 5 days of culture for the b-amyloid protein peptides and Flu. Production of IL-2 and IL-10 by PBMCs was evaluated with commercial available ELISA kits (ACCUCYTE, Cytimmune Sciences, Inc, College Park, MD). All test kits were used following the procedures suggested by the manufacturer.

IL-10 genotyping

Genomic DNA was extracted from EDTA-treated peripheral blood (10 ml) using a standard proteinase K and phenol/chloroform method. The DNA concentration and purity were determined by spectrophotometric analysis. A polymerase chain reaction-sequence specific primers (PCR-SSP) methodology was utilised to assess the IL-10 genotypes. The amplification of the sequence in the promoter region of the IL-10 (polymorphic positions -1082, -819, -592) gene were performed using the Cytokine genotyping Tray Method (One Lambda, Canoga Park, CA, USA); the human b-globin gene was amplified as an internal control of genomic DNA preparation. PCR

condition were indicated by One Lambda PCR program (OLI-1); the PCR products were then visualised by electrophoresis in 2.5% agarose gel.

Statistical analysis

Statistical analysis was conducted using SPSS statistical package (SPSS, Chicago, IL). Differences in IL-10 production stemmed from analytic procedures based on non parametric analyses (Mann-Whitney); comparisons between different groups of patients were made using Fisher's exact 2-tailed test. Genotype frequencies were compared between the study groups by c^2 test with an observed significance level of the test below 0.05. Comparisons between the mean values of the age at onset and MMSE in the six different groups of AD were performed by one-way ANOVA analysis.

Age, gender and MMSE scores in AD patients and in HC

Forty-seven AD patients and 25 age-matched healthy controls were enrolled in the study. The Mini-Mental State Evaluation (MMSE) showed the presence of a mild-to-severe cognitive deterioration in the AD patients. These data are shown in Table I.

MBP-stimulated IL-10 production is reduced in AD patients

PBMC of 47 AD patients and of 25 age-and sex-matched HC were stimulated with a mitogen (LPS); a pool of 3 amyloid peptides (A: fragment 25-35, B: fragment 1-40, and C: fragment 1-16)(amyloid), or Flu (used as a control antigen) and the production of IL-2 and IL-10 was measured with ELISA methods. No differences were seen when LPS- or Flu-stimulated IL-2 and IL-10 production was compared in AD patients and HC. amyloid-stimulated IL-2-production was also similar in the two groups of individuals studied. In contrast with these results, amyloid-stimulated production of IL-10 was significantly reduced (p= 0. 023) in AD patients compared to controls. These data are shown in Figure 1.

The distribution of high, intermediate, and low IL-10 producing genotypes is skewed in AD patients

Paradigmatic example of the six different IL-10 genotypes, evaluated by PCR-SSP, is showed in Fig. 2 and their relative distribution among a typical caucasic population sample is shown in Table II. In contrast with the distribution observed in HC, the frequency of the different IL-10 genotypes among AD patients was significantly skewed (c^2 = 16.007 with p=0.007) (Table II). Therefore genotypes corresponding to reduced IL-10 production (ACC/ACC, ACC/ATA and ATA/ATA genotypes) had a significantly higher distribution amongst AD subjects (17%, 26% and 11% respectively versus 4%, 16% and 4% in HC). Moreover the GCC/ACC to GCC/ATA ratio (intermediate phenotype) was 1:1 in AD while was 3:1 in HC.

Low IL-10 production is correlated with worsened clinical outcome of AD

To analyse possible clinical correlates of the presence of low IL-10 genotype, we subsequently examined the six genotypes in relation to age of AD onset (Table III) and the progression of cognitive deterioration (Table IV). The results confirmed that the presence of low-IL-10-producing genotypes is indeed associated with a worsened clinical outcome of AD. Thus, presence of the ATA/ATA and GCC/ATA genotypes was associated with an earlier age at disease onset (ANOVA: p=0.042)(Table III); additionally, an inverse correlation was detected between ACC/ATA and ACC/ACC, low IL-10-producing genotypes, and the MMSE score (ANOVA: p=0.010)(Table IV).

Figure 1. LPS- and amyloid- (a pool of 3 amyloid peptides: A: fragment 25-35; B: fragment 1-40; and C: fragment 1-16) stimulated IL-2 (panels A and C) and IL-10 (panels B and D) production by PBMC of 47 AD patients (O) and 25 age-and sex-matched healthy controls (O). Mean values + standard errors are shown. $p \leq 0.05$.

Figure 2. Paradigmatic example of IL-10 genotyping for six different samples. In each gel the heaviest bands correspond to the amplicons of the human b-globin gene which is used as the internal controls. The other specific amplified DNA fragments correspond to the polymorphisms of the IL-10 gene:

Genetic Association Data for Autoimmune/Inflammatory Disease

www.grc.nia.nih.gov/branches/rrb/dna/geneticdata.htm

Chrom	1st Band	Gene	Disease	Allele	P-value	Reference	PubMed ID
1	1q31.1	CD45	Ms	C to G in position 77 of PTPRC exon 4.	P=1.510-4	Jacobsen M 00	11101853
1	1q31.1	CD45	SCId	deletion	na	Kung C 00	10700239
	mouse	CD45	autoimmune nephritis	glutamate 613 to arginine	na	Majeti R 00	11163182
1	1q32.1	IL10	SLE	-4kb to 5'	P=.0001	Gibson AW 01	11238636
1	1q32.1	IL10	SS	-10 GCC haplotype (G -1082, C -819, and C -592 of the IL-10 gene	P=<0.05	Hulkkonen J 01	11212157
1	1q32.1	IL10	RA	genotype -1082GG	P=<0.03	Huizinga TW 00	11085795
1	1q32.1	IL10	RA	ATA haplotype, pts w/>4 joints	P=0.02	Crawley E 99	10366102
1	1q32.1	IL10	GVHD	IL-10 (-)1064	P=<.001	Middleton PG 98	9808588
1	1q32.1	IL10	IBD/UC	-1082*G allele (high producer) was reduced in pts	P=0.03	Tagore A 99	10551422
2	2q12.2	IL1RA	SLE	IL1RN*2 allele	na	Blakemore AL 94	7945503
2	2q12.2	IL1RA	Ulcerative	IL1RN*2 allele	P=0.007	Mansfield JC 94	8119534

			Colitis				
2	2q12.2	IL1RA	polymyalagia rheumatica	IL1RN*2 allele	na	Boiardi L 00	11138328
2	2q33.1	CTLA4	RA	A/G 49	P=0.009	Gonzalez MF 99	10203024
2	2q33.1	CTLA4	GD	A/G 49	P=<0.01	Yanagawa T 97	9459626
2	2q33.1	CTLA4	MS	A/G 49	P=0.006	Harbo HF 99	10082437
2	2q33.1	CTLA4	H-Thy	A/G 49	P=<0.03	Donner H 97	9398726
2	2q33.1	CTLA4	IDDM	A/G 49	P=0.004	Takahiro A 99	
2	2q33.1	CTLA4	IDDM		na	Yanagawa T 99	10052685
2	2q33.1	CTLA4	IDDM	A/G 49	P=0.00002	Marron MP 97	9259273
5	5q31.1	IL4	GD	position 590 allele reduced in GD	P=0.00004	Hunt PJ 00	10843185
5	5q31.1	IL4	increased IgE	C+33T polymorphism with elevated total serum IgE	P=<0.05	Suzuki I 00	11122213
5	5q31.1	IL4	asthma, FEV(1)	C-589T IL-4 promoter genotype (TT)	P=0.013	Burchard EG 99	10471619
5	5q31.1	IL4	AD	-590C/T	P=0.001	Kawashima T 98	9643293
5	5q31.1	IL4	RA	IL-4(2) higher in non-destructive RA	P=0.0006	Buchs N 00	11035134
5	5q31.1	IL4	MS	IL-4 B1 allele, late onset MS	P=<0.001	Vandenbroeck K 97	9184650

5	5q31.1	IL13	asthma	Gln110Arg	P=0.017	Heinzmann A 00	10699178
5	5q31.1	IL13	asthma	C to T at position -1055 (TT)	P=0.002	van der Pouw Kraan TC 99	11197307
6	6p21.31	TNFa	asthma	G/A -308 TNF2	P=0.003	Albuquerque R 98	9645594
6	6p21.31	TNFa	PrimBilCirr	G/A -308 TNF1	P=0.02	Gordon M 99	10453936
6	6p21.31	TNFa	Sepsis	G/A -308 TNF2	P=0.007	Majetschak M 99	10450735
6	6p21.31	TNFa	Psoriasis	G/A -308 TNF1	P=2.74 X 10 -8	Arias A 97	9395887
6	6p21.31	TNFa	lep. Leprosy	G/A -308	P=.02	Roy S 97	9237725
6	6p21.31	TNFa	GVHD	TNFd	P=.006	Middleton PG 98	9808588
6	6p21.31	TNFa	Silicosis	G/A -308 TNF1	P=<0.05	Yucesoy B 01	11264025
6	6p21.31	TNFa	SLE	G/A -308 TNF1	na	Sullivan KE 97	9416858
6	6p21.31	TNFa	celiac	G/A -308 TNF1	P=<0.001	McManus R 96	8655356
6	6p21.31	TNFa	chronic bronchitis	G/A -308 TNF1	P=<0.01	Huang S 97	9372657
6	6p21.31	TNFa	Psoriasis	-238 TNF1	P=1.64 X 10 -7	Arias A 97	9395887
7	7p15.3	IL6	IDDM	G,G(-174) increased in pts	P=<0.002	Jahromi MM 00	11054276

7	7p15.3	IL6	SLE	AT-rich minisatellite in 3' flanking-region	P=0.001	Linker-Israeli M 99	11197305
7	7p15.3	IL6	RA	622 and -174 alleles, age of onset	na	Pascual M 00	11196696
7	7p15.3	IL6	MS	carriage larger alleles A6-->A9, accelerated onset	P=0.025		
12	12q12	VDR	GD	exon 2 initiation codon (VDR-FOK:I) polymorphism	P=0.023	Ban Y 00	11134121
12	12q12	VDR	RA	BB/tt genotype	na	Garcia-Lozano JR 01	11251690
12	12q12	VDR	MS	bb	P=0.0263	Fukazawa T 00	10465499
12	12q12	VDR	CD	tt	P=0.017	Simmons JD 00	10896912
12	12q12	VDR	IDDM	BsmI	P=0.015	Chang TJ 00	10792336
12	12q21.1	IFNG	asthma	CA repeat polymorphism within the first intron	P=.0018	Nakao F 01	11240951
12	12q21.1	IFNG	IDDM	CA repeat polymorphism within the first intron	P=0.039	Awata T 94	7867888
12	12q21.1	IFNG	GD	CA repeat polymorphism within the first intron	P=<0.04	Siegmund T 98	9848715
12	12q21.1	IFNG	RA	CA repeat polymorphism within the first intron	P=<0.0001	Khani-Hanjani A 00	11022930
16	16p11.1	IL4R	asthma	Ile50Val	P=<0.0001	Mitsuyasu H 98	9620765

16	16p11.1	IL4R	hyper-IgE syndrome and severe eczema, atopy	Arg576G	P=0.001	Hershey GKK 97	9392697	
16	16p11.1	IL4R	MS(PPMS)	IL4R variant:R551	P=0.001	Hackstein H 01	11164908	

[DNA Array Unit] [IRP Home] [NIA Home]

References

1. Ernst, R.L. and J.W. Hay. 1994. The US economic and social costs of Alzheimer's disease revised. *Am. J. Public Health*. 84: 1261.

2. Goate, A., M.C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James, R. Mant, P. Newton, K. Rook, P. Roques, C. Talbot, M. Pericak-Vance, A. Roses, R. Williamson, M. Rossor, M. Owen and J. Hardy.1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* . 349: 704.

3. Levy-Lahad, E., W. Wasco, P. Poorkaj, D.M. Romano, J. Oshima, W.H. Pettingell, C.E. Yu, P.D. Jondro, S.D. Schmidt, K. Wang, A.C. Crowley, Y.H. Fu, S.Y. Guenette, D. Galas, E. Nemens, E.M. Wiisman, T.D. Bird, G.D. Schellemberg and R.E. Tanzi. 1995. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* . 269: 973.

4. Sherrington, R., E.I. Rogaev, Y. Liang, E.A. Rogaeva, G. Levesque, M. Uveda, H. Chi, C. Lin, G. Li, K. Holman, T. Tsuda, L. Mar, J.F. Fonci, A.C. Bruni, M.P. Montesi, S. sorbi, I. Rainero, L. Pinessi, L. Nee, I. Chumaken, D. Pollen, A. Brookes, P. Sanseau, R.J. Polinsky, W. Wasco, H.A.R. Da Silva, J.L. Haines, M.A. Pericak-Vance R.E. Tanzi, A.D. Roses, P.E. Fraser, J.M. Rommens and P.H. George-Hyslop. 1995. Cloning of a gene bearing missense mutation in early-onset familial Alzheimer's disease. *Nature*. 375: 754.

5. Blacker, D., J.L. Haines, L. Rodes, H. Terwedow, R.C.P. Go, L.E. Harrel, R.T. Perry, S.S. Basset, G. Chase, D. Meyers, M.S. Albert and R. Tanzi. 1997. ApoE-4 and age at onset of Alzheimer's disease: The NINH Genetics Initiative. *Neurology*.. 48: 139.

6. Poirier, J., J. Davignon, D. Bouthillier, S. Kogan, p. Bertrand and S. Gauthier. 1993. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet.* 342: 697.

7. Saunders, A.M., W.J. Strittmatter, D. Schmechel, P.H. George-Hyslop, M.A. Pericak-Vance, S.H. Joo, B.L. Rosi, J.F. Gusella, D.R. Crapper-MacLachlan and M.J. Alberts.1993. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology.*. 43:1467.

8. Fassbender, K., C. Masters and K. Beyreuther.2000. Alzheimer's disease: an inflammatory disease?. *Neurobiology of Aging.* 21: 433.

9. McGeer, P.L. and E.G. McGeer. 2001. Inflammation, autotoxicity and Alzheimer disease. *Neurobiology of Aging.* 22: 799.

10. Zandi, P.P and J.C.S. Breitner. 2001. Do NSAIDs prevent Alzheimer's disease? And, if so, why? The epidemiological evidence. *Neurobiology of Aging.* 22: 811.

11. Rogers, J., L.C: Kirby, S.R. Hempelman, D.L. Berry, P.L. McGeer, A.W. KasniaK, j. Zalinski, M. Cofield, L. Mansukhani, and P. Willson.1993. Clinical trial of indomethacin in Alzheimer's disease. *Neurology.* 43: 1609.

12. Hauss-Wegrzyniak, B., L.B. Willard, P. Del Soldato, G. Pepeu and G.L. Wenk. 1999. Peripheral administration of novel anti-inflammatories can attenuate the effects of chronic inflammation within the CNS. *Brain Res.* 815: 36.

13. Neuroinflammation Working Group. 2000. Inflammation and Alzheimer's disese. *Neurobiology of Aging.* 21: 383.

21. Engelborghs, S., M. De Brabander, J. De Cree, R. D'Hooge, H. Geerts, H. Verhaegen and P.P. De Deyn. 1999. Unchanged levels of interleukins, neopterin, interferon-gamma and tumour necrosis factor-alpha in cerebrospinal fluid of patients with dementia of the Alzheimer type. 34: 523.

22. Hutchinson, I.V., V. Pravica and P.J. Sinnot . 1998. Genetic regulation of cytokine synthesis: consequences or acute and chronic organ allograft rejection. *Graft.* 1: 15.

23. McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Proce, E.M. Stadlan. 1984. Clinical diagnosis of Alzheimer's disease. *Neurology.* 34: 939.

24. Eikelenboom, P., S.S. Zhan, W.A. van Goll and D. Allsop D. 1994. Inflammatory mechanisms in Alzheimer disease. *Trends Pharmacol Sci* 15:447.

25. Rogers, J., S. Webster, and L.F. Lue. 1996. Inflammation and Alzheimer's disease pathogenesis. *Neurobiol Aging* 17:686.

26. Llorente, L., W. Zou, Y. Levy, Y. Richaud-Patin, Y. Wijdenes, J. Alcocer-Varela, B. Morel-Fourrier, J.C. Brouet, D. Alarcon-Segovia, P. Galanaud. 1995. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythemattosus. *J. Exp. Med.* 181: 839.

27. Luscher, U., L. Filgueira, A. Juretic, M.Zuber, L.J. Luuscher, M. Heberer and g.C. Spagnoli. 1994. The pattern of cytokine gene expression in freshly excised human metastatic melanoma suggests a state of reversible anergy of tumor-infiltrating lymphocytes. *Int. J. Cancer.* 57: 612.

28. Matsuda, M., F. Slazar, M. Petersson, G. Masucci, J. Hansson, Q.C. Zhang, M.G. Masucci and R. Kiessling. 1994. Interleukin 10 pretreatment protects target cells from tumor- and allo-specific cytottoxic T cells and downregulates HLA class I expression. *J. Exp. Med* .180: 2371.

29. Kim, J., R.L. Modlin, R.L. Moy, S.M. Dubinett, T. McHugh, B.J. Nickloff and K. Uyemura. 1995. IL-10 production in cutaneous basal and squamos cell carcinomas. A mechanism for evading the local T cell immune response.*J. Immunol.* 155: 2240.

30. Suzuki, T., H. Tahara, S. Narula, K.W. Moore, P.D. Robbins, and M.T. Lotze. 1995. Viral interleukin 10 (IL-10), the human herpes virus 4 cellular IL-10 homologue, induces local anergy to allogenic and syngenic tumors. *J. Exp. Med.* 182: 447.

31. Fortis, C., M. Foppoli, L. Gianotti, L. Galli, G. Citterio, G. Consogno, O. Gentilini and M. Braga. 1196. Increased interleukin-10 serum levels in patients with solid tumours.*Cancer Lett.* 104: 1.

32. Murray, P.J., L. Wang, R.C. Onufry, R.I. Tepper,and R.A. Young. 1997. T cell-derived IL-10 antagononlzes macrophage function in mycobacterial infection. *J. Immunol.* 158: 315.

33. Lehmann, A.K., A. Halstenen, S. Somes, O. Rokkeand A. Waage. 1995. High levels of interleukin 10 in serum are associated with fatality in meningococcal disease. *Infect. Immun.* 63: 2109.

34. Clerici, M., T.A. Wynn, J.A. Berzofsky, R.L. Coffman, A. Sher, G.M. Shearer. 1994. Role of Interleukin-10 (IL-10) in T Helper Cell Dysfunction in Asymptomatic Individuals Infected with the Human Immunodeficiency Virus (HIV-1). *J. Clin. Invest.* 93:768.

35. VanFurth, A.M., E.M. Seijmonsbergen, J.A.M. Langermans, P.H.P. Groeneveld, C.E. Debel and R. VanFurth. 1995. High levels of interleukin 10 and tumor necrosis factor alpha in cerebrospinal fluid during the onset of bacterial meningite. *Clin. Infect. Dis.* 21: 220.

36. Llorente, L., Y. Richaud-Patin, J. Couderc, D. Alarcon-Segovia, R. Ruiz-Soto, N. Alcocer-Castillejos, J. Alcocer-Varela, J. Granados, S. Bahena, P. Galanaud and D. Emilia. 1997. Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. *Arthritis Rheum.* 38: 1429.

37. Westendorp, R.G.J., J.A.M. Langermans, T.W.G. Hulzinga, A.H. Elouali, C.L. Verwej and J.P. Vandenbroucke. 1997. Genetic influence on cytokine production and fatal meningococcus disease. *Lancet.* 349:170.

38. Eskdale, J., G. gallagher, C.L. Vermeij, V. Keijsers, R.G.J. Westendorp and T.W.J. Hulzinga. 1998. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc. Natl. Acad. Sci. USA.* 95: 9465-9470.

39. Derkx, B., A. marchant, M. Goldman, R. Billmer and S. Van de Venter. 1995. High levels of interleukin-10 during the initial phase of fulminant meningococcal septic shock. *J. Infect. Dis.* 171: 229.

40. Llorente, L., Y. Richaud-Patin, R. Fior, J. Alcocer-Varela, J. Wijdnes, B. Morel-Fourrier, P. Galanaud and P. Emilie. 1994. In vivo production of interleukin-10 by non -T cells in rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus. A potential mechanism of B lymphocytes hyperactivity and autoimmunity. *Arthritis Rheum.* 37: 1647.

41. Cash, J.J., J.B. Splawski, R. Thomas, J.F. McFarlin, H. Schulze-Koops, L.S. Davis, K. Fujita and P.E. Lipsky. 1995. Elevated interleukin-10 levels in patients with rheumatoid arthritis. *Arthritis Rheum.* 38: 96.

42. Eskdale, J. P. Wordsworth, S. Bowman, M. Field and G. Gallagher. 1997. Association between polymorphisms at the human IL-10 locus and systemic lupus erythematosus. *Tissue Antigens.* 49: 635.

43. Zheng, C., D. Huang, L. Liu, R. Wu, S. Bergenbrant Glas, A. Ostenborg, M. Bjorkholm, G. Holm, Q. Yi, A. Sundblad. 2001. Interleukin-10 gene promoter polymorphisms in multiple myeloma. *Int. J. Cancer.* 95: 184.

44. Via, C.S., G.C.Tsokos, N.I. Stocks, M. Clerici and G.M. Shearer. 1990. Human in vitro allogeneic responses: demonstration of three pathways of T helper cell activation. *J. Immunol.* 144:2524.

45. Hahn, A.B., J.C. kasten-Jolly, D. M. Costantino, E. Graffunder, T.P. Singh, G.K. Shen and D.J. Contl. 2001. Tnf-a, Il-6, IFN-g, and IL-10 gene expression polymorphisms and the IL-4 receptor a-chain variant Q576R: effects on renal allograft outcome. *Transplantation.*. 72: 660.

46. Lio, D., G. Candore, A. Colombo, G. Colonna Romano, F. Gervasi, V. Marino, L. Scola and C. Caruso. 2001. A genetically determined high setting of TNF-alpha influences immunologic parameters of HLA-B8, DR3 positive subjects: implications for autoimmunity. *Hum Immunol.* 62: 705.

47. Akdis, C.A., and K. Blaser K. 2001. Mechanisms of interleukin-10-mediated immune suppression. *Immunol.* 103:131.

48. Segal, B.M., B.K. Dwyer and E.M. Shevach. 1998. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease *J Exp Med* 187: 537.

CLAIMS

1. Use of cytokines in the preparation of a medicament for the treatment or prophylaxis of diseases which are not neoplastic.

2. Use according to claim 1, characterised in that the disease is a neurodegenerative disorder or an autoimmune disorder.

3. Use according to claim 1 or claim 2, characterised in that the use is for Alzheimer's disease.

4. Use according to any one of claims 1 to 3, characterised in that the cytokines is selected from interleukin-1 (α or β), interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, interleukin-17, interferon-α, interferon-β, interferon-γ, TNF-α, TNF-β, G-CSF, GM-CSF, M-LSF, and TGF-β.

5. A method of determining a predisposition to Alzheimer's disease, autoimmune disease or other neurodegenerative diseases, the method comprising taking a DNA bearing sample from a subject animal, analysing the sample for the presence or absence of the alleles of Figure 2.

6. A method of treating Alzheimer's disease, autoimmune disease or other neurodegenerative disorder by the upregulation of one of the allelic polymorphisms of Figure 2.

7. A method of treating Alzheimer's disease, autoimmune disease or other neurodegenerative disorder by the downregulation of one of the allelic polymorphisms of Figure 2.

8. DNA fragments and cDNA fragments encoding the allelic polymorphs of Figure 2 for use in the method of claim 5.

ABSTRACT

An inflammatory process is suggested to be involved in the pathogenesis of Alzheimer's disease (AD), a neurodegenerative disorder characterized by the presence of neuritic plaques within the cerebral cortex that are mainly composed of a small insoluble protein of 40-42 aminoacids (amyloid protein). The biological correlates of this process are nevertheless not clear. Interleukin-10 (IL-10) is a cytokine that suppresses T lymphocytes and cell-mediated immunity in humans and mice and has potent anti-inflammatory properties. To verify if IL-10 production would be impaired in AD patients we stimulated PBMC of 47 patients and 25 age-matched healthy controls (HC) with a mitogen, a recall antigen or with amyloid peptides. IL-2 production was measured as well in the same cultural conditions. Results showed that amyloid-specific IL-10 generation is selectively and significantly reduced in AD patients ($p = 0.023$). Analyses on the alleles of the IL-10 gene revealed that the genotype associated with high IL-10 production is extremely infrequent in AD individuals (2% vs. 28%). The presence of low/intermediate-IL-10-producing genotypes (GCC/ATA; ATA/ATA) was associated with an earlier age at disease onset and (ACC/ACC; ACC/ATA) with an accelerated rate of disease progression. These data shed light on the biology of the inflammatory process involved in the pathogenesis of AD by showing that the presence of low-IL-10-allelic isoforms results in an amyloid-specific impairment of IL-10 production and is associated with the clinical severity of AD. These results lend support to the use of anti-inflammatory compounds in the therapy of this disease.